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16. ABSTRACT

Alternative Methods of Disinfection - The public health issues which have arisen around the disinfection of drinking water have become quite complex. Historically, disinfection has been viewed as the mainstay in preventing waterborne infectious disease. Chlorine has been the principle agent used in the United States for this purpose. The use of chlorine was first questioned as a result of the observation that it resulted in the formation of trihalomethanes. Shortly following this finding, a National Cancer Institute study demonstrated that chloroform, or trichloromethane, produced an increased tumor incidence in both mice and rats.

This article will focus on reviewing progress that has been made in the last several years in identifying toxicological hazards that are associated with alternate disinfectants themselves or with their reaction products. As a point of departure, this review will concentrate on research results available since a prior review of the area.

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Toxicological problems associated with alternative methods of disinfection

Richard J. Bull

In a review of research results available since a previous review in 1980, the author discusses the toxicological hazards associated with alternative means of disinfecting drinking water. These hazards can be associated with the disinfectants themselves as well as with the products formed when disinfectants react with organic material present in the water. Effects of disinfectants—chlorine, chlorine dioxide, and monochloramine—and by-products—chlorite, chlorate, trihalomethanes, and haloacetonitriles—are considered.

The public health issues that have arisen around the disinfection of drinking water have become quite complex. Historically, disinfection has been viewed as the mainstay in preventing waterborne infectious disease. Chlorine has been the principal agent used in the United States for this purpose. The use of chlorine was first questioned as a result of the observation that chlorination resulted in the formation of trihalomethanes (THMs).[1,2] Shortly following this finding, a study by the National Cancer Institute (NCI) demonstrated that chloroform—trichloromethane—produced an increased incidence of tumors in both mice and rats.[3]

The first reaction to these observations was to examine alternative means of disinfection that would not give rise to the trihalomethanes. Soon it became apparent that very little was known about potential hazards associated with commonly mentioned alternatives. Moreover, all common and cost-effective means of disinfecting drinking water involve the use of reactive chemicals, usually oxidants. As a consequence, judgments concerning the safety of alternative means of disinfection must focus not only on the disinfectants but also on hazards associated with products of the reactions between disinfectants and background organic chemicals that occur in every water drawn from surface sources and from some groundwaters.

This problem is further exacerbated by the poor characterization of the organic chemicals present in sources of drinking water. Much attention has focused on the great diversity of chemicals that can be identified by gas chromatography-mass spectrometry (GC-MS), but it has not been generally appreciated that these chemicals account for less than 10 percent of the organic material actually present in surface waters. It is difficult to predict and thus measure products of reactions between disinfectants and unknown substrates.

Within the last few years, research to define potential and often competing health hazards associated with alternate disinfectants has taken a much broader view of the problem. The primary concerns revolve around three major issues: (1) efficacy of a disinfectant, (2) toxic effects of the disinfectant, and (3) toxic effects of its reaction products.

Discussing the efficacy of disinfectants is beyond the scope of this article. However, it should be made clear that efficacy of a disinfection process involves not only questions of bactericidal properties but also requirements for distributing a microbiologically safe water. Within the United States, conventional wisdom (and in some cases state or local codes) provides for the maintenance of residual disinfectant in the distribution system. Alteration of this generally accepted rule would represent a substantial change in the philosophy of treating drinking water. Although the efficacy of different disinfectants is not specifically addressed in this article, the reader should keep in mind that the overriding issue is the prevention of waterborne infectious disease.

The present article reviews progress that has been made in the last several years in identifying toxicological hazards that are associated with alternate disinfectants themselves or with their reaction products. As a point of departure, this review concentrates on research results available since a previous review.[4]

Toxicological effects of disinfectants

Early work in this area focused on the hematological effects known to be associated with chlorine dioxide (ClO_2) and its inorganic by-products—chlorite (ClO_2^-) and chlorate (ClO_3^-). The early work of Musil et al[5] had established that ClO_2^- was capable of producing methemoglobinemia. Work conducted at the Health Effects Research Laboratory (HERL) of the US Environmental Protection Agency in Cincinnati established that ClO_2^- produced hemolytic anemia and that these effects occurred at exposures less than those required to produce significant

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increases in methemoglobin.[6,7] Other work sponsored by HERL at Ohio State University, the University of Massachusetts, and the College of Medicine and Dentistry of New Jersey extended these observations to ClO_2 and ClO_3^-, although ClO_2^- remained the more potent chemical for producing signs of hemolytic anemia. These effects were found consistently across several species, although the sensitivity to the effects varied somewhat.[8]

Currently, the Toxicology and Microbiology Division (TMD) of HERL is investigating the effects of ClO_2 on thyroid function.[9] Results from experiments conducted with African green monkeys are depicted in Table 1. In these experiments, the dosage of ClO_2 was increased in a stepwise fashion, allowing each animal to serve as its own control, both at the start of the experiment before exposure to ClO_2 and at the end of an 8-week recovery period at the end of the experiment. These experiments showed that a 4-week exposure to 30 mg ClO_2/L was without significant effect on serum thyroxine levels. However, within 1 week after the level of exposure was increased to 100 mg/L, serum thyroxine levels fell. After 4 weeks of treatment at 100 mg/L, the levels of thyroxine declined to less than 75 percent of the initial levels. Eight weeks after exposure to ClO_2 was ended, serum thyroxine levels recovered to their original values. Exposing African green monkeys to 400 mg ClO_2^-/L for 6 weeks reduced serum thyroxine levels by only 12 percent, and equivalent exposures to ClO_3^- actually increased serum thyroxine levels slightly. Another disinfectant—monochloramine (NH_2Cl)—also failed to produce significant alterations of serum thyroxine levels.

The above experiments have been repeated with rats, giving results that were similar to those obtained in the experiments with primates.[10] Preliminary experiments indicate that antithyroid effects of ClO_2 could affect the normal development of rat pups.[11]

These data have several important implications for the use of ClO_2 as a primary disinfectant. It is clear from the above discussion that the effects of ClO_2 on thyroid function are independent of its two major, inorganic by-products—ClO_2^- and ClO_3^-. On the other hand, the role of ClO_2 in producing hemolytic anemia is apparently dependent on its conversion to ClO_2^-. Thus, it is apparent that if ClO_2 is to be used as a disinfectant, limits will have to be placed on its concentration in the finished water. Moreover, consideration will have to be given to the formation of ClO_2^-. This may severely limit or even prevent the use of ClO_2 in treating waters with a high demand for disinfectant.

To date, there are few data to suggest that there are any direct effects of chlorine that would limit its use as a primary disinfectant. Chlorine appears to possess none of the activity of ClO_2^- in producing hemolytic anemia.[8] As mentioned, chlorine does not appear to possess the antithyroid effects of ClO_2. Druckrery[12] was able to maintain rats on 100 mg chlorine/L of drinking water for seven generations without observing any adverse effects. Similar levels of ClO_2 shortened the life span of rats significantly.[13] One report indicated that consumption of water containing 25 mg chlorine/L did affect the function of peritoneal macrophages in mice; however, no adverse health outcome was associated with this depressed function.[14]

Some concern must be expressed over potential .indirect effects of chlorine. Recent data have shown that administering high doses of chlorine to rats results in the formation of chloroform (found in blood and contents of stomach).[15] Recent experiments in TMD have confirmed these experiments and also documented the formation of dichloroacetic acid and trichloroacetic acid under similar circumstances in fasted rats.[16] In addition, dichloroacetonitrile has been found in the stomachs of rats that have not been fasted. Potential hazards of these products are dealt with in subsequent sections of this article.

Using NH_2Cl as a disinfectant for drinking water has attracted attention because production of THMs is considerably decreased when NH_2Cl is used instead of chlorine. However, NH_2Cl has been shown to be mutagenic in *Bacillus subtilis*.[17] On the basis of this observation, the National Toxicology Program (NTP) has initiated studies of NH_2Cl's carcinogenic activity in lifetime bioassays with rats and mice. Results from the 90-day, range-finding studies have indicated increased mitotic figures, bizarre chromatin patterns, and increased cell size in the liver of the B6C3F1 mouse.[18] Such changes are often associated with chemicals that produce cancer in the liver. Resolution of this problem will have to await completion of the lifetime studies.

Chloramine has also been shown to be capable of oxidizing hemoglobin to methemoglobin in vitro. This property of NH_2Cl has been responsible for producing hemolytic anemia in patients undergoing dialysis.[19] However, no indication of hemolytic anemia has been found in animals consuming NH_2Cl by the oral route at concentrations as high as 100 mg/L.[4]

A series of pilot experiments have been conducted to assess the teratogenic effects of disinfectants.[20,21] These experiments failed to demonstrate statistically significant increases in fetal abnormalities. However, the available data indicate an increase in skeletal anomalies (from 34.5 percent in controls to 59.1 percent in animals treated with 10 mg hypochlorous acid [HOCl]/L) and an increase in anomalies of soft tissue (from 7.1 to 19.2 percent in animals treated with 100 mg HOCl/L).[20] However, the difference in total defects (skeletal and soft tissue) appeared to approach statistical significance. Similar trends were observed with ClO_2 and ClO_2^-.[21] Unfortunately, the number of animals used in these studies was insufficient to distinguish between even these large differences in the incidence of anomalies. As a consequence of the limited nature of the data, it is not possible to arrive at any firm conclusions with respect to teratogenic activity of the alternate disinfectants.

Preliminary evaluations of the effects of disinfectants have been made directly in human male volunteers. Branchine et al[22] examined the effects of increasing doses of ClO_2, ClO_2^-, ClO_3^-, NH_2Cl, and chlorine in single acute doses of up to 24 mg/L in a volume of 500 mL. In addition, volunteers were given 500 mL of distilled water containing 5 mg/L of each of the disinfectants or their inorganic by-products daily for 20 weeks. Although there were some statistically significant trends in clinical parameters associated with these treatments, none fell outside the normal range. No signs of the hemolytic anemia observed in experimental animals with ClO_2 and ClO_2^- were observed in

TABLE 1
*Effects of chlorine dioxide and its by-products and monochloramine on thyroxine levels of primates**

Disinfectant or By-Product	Concentration in Drinking Water—mg/L (Weeks on Treatment)					
	0	30 (4)	100 (1)	100 (4)	400 (6)	0 (8)‡
ClO_2	4.7†	4.9	4.4	3.5		5.3
ClO_2^-	5.0				4.4	5.3
ClO_3^-	5.3				5.7	
NH_2Cl	5.3			5.8		

*Adapted from Bercz et al[9]
†Serum thyroxine (T_4) levels in μg/dL
‡8-week recovery

this work. However, only a limited evaluation was made with individuals of genetic types that are known to be extremely sensitive to hemolytic agents. In vitro studies have shown that human hemoglobin is as sensitive as animal hemoglobin[6] to the hematological effects of ClO_2^- and that blood from individuals with a glucose-6-phosphate dehydrogenase deficiency is much more sensitive than that from normal individuals.[23] Although these data do not necessarily establish the safety of the disinfectants for longer periods of time or for sensitive populations, they do indicate that the disinfectants are not grossly toxic to normal, healthy human males at levels of exposure in the range likely to be encountered as a result of drinking disinfected water. Substantive changes in present practices of disinfecting drinking water should not be contemplated without monitoring those segments of the population that may be more sensitive to the disinfectant than normal, healthy human males. Data from animal studies with ClO_2 indicate that individuals with impaired thyroid function or with greater than normal susceptibility to hemolytic agents are of particular concern.

Toxicology of organic by-products

Following the observation that chloroform produced cancer in experimental animals, several epidemiological studies were undertaken to determine whether any associations could be established between the chlorination of drinking water and the incidence of various cancers in the human population. Many of these studies did find simple correlations between incidence of cancer and chlorination of drinking water. These early studies were not definitive in that most were small-scale and did not take into account a variety of potentially confounding factors. The National Academy of Sciences concluded that these studies did not provide substantive evidence for a relationship between incidence of cancer and chlorination of drinking water.[24] On the other hand, more recent and sophisticated studies have confirmed the results of these simple correlational studies.[25-29] These recent studies still do not conclusively prove a causal relationship between chlorination of drinking water and incidence of cancer. However, they do provide a substantial reason for continuing research efforts in order to eventually decide this issue. Although the differences implied in incidences of cancer are small, chlorination of drinking water is widely enough practiced that remedial action could have a substantial impact on the number of cases of cancer.

Judging hazards that might be associated with by-products of the reactions between disinfectants and background organic material is quite complex. Although certain chemicals have been clearly identified as being associated with the use of a disinfectant, the qualitative and quantitative nature of by-product formation is very dependent on characteristics of the water being studied. Model studies of interactions between disinfectants and pure chemicals or complex mixtures (e.g., humic acid) have been useful in providing a base of information. However, it is clear that studies to date fall far short of identifying even a majority of the by-products formed. It is considerably beyond the scope of this article to deal with the complexities associated with the chemistry of disinfectants and the characteristics of the substrates involved. Therefore, the discussion of by-products will be limited to: (1) the toxicology of individual chemicals that have been consistently identified as by-products of disinfectants, and (2) important chemical and biological properties of unidentified by-products that are produced when disinfectants are applied to complex mixtures of organic chemicals.

Organic by-products of disinfection

Various chemicals have been identified as by-products of reactions between disinfectants and different organic substrates. Few of these by-products have been consistently identified in actual drinking waters and even fewer have been studied enough to allow estimation of the hazards they present to human health in qualitative and quantitative terms.

The THMs remain the most consistently identified by-products associated with the use of chlorine as a disinfectant. Chloroform and the other THMs produce definite toxicological effects in the liver and kidney;[30] however, at the concentrations in which these chemicals are found in drinking water, their role in producing cancer remains the most critical issue. Chloroform is the only THM that has been shown to be carcinogenic to date.[3] Bromoform, dichlorobromomethane, and dibromochloromethane are currently being tested in the NTP. These studies should be completed in 1983. Unlike chloroform, these three THMs have been identified as mutagens in *Salmonella*.[31]

Considerable debate has surfaced in the scientific literature as to the mechanism by which chloroform and certain other chlorinated hydrocarbons produce cancer.[32] In recent years it has become evident that many chemical carcinogens interact with the genetic material (DNA) of cells. As a result of this discovery, many scientists have come to believe that cancer arises from a single cell with altered DNA so that it and its daughter cells have an altered genotype with respect to the surrounding normal cells. This means of explaining how cancer can arise is referred to as the somatic mutation theory of chemical carcinogenesis. One consequence of this theory is that the probability of producing cancer is directly related (in a linear fashion) to the dose at low response rates (i.e., there is no threshold for the effect of a chemical carcinogen). However, it has long been known that chemically induced cancer involves at least two distinct stages. These stages are classically termed initiation and promotion. Initiation would correspond to an irreversible process such as a cell mutation. This effect can result from a single exposure to an initiator. Consequently, it is an irreversible event and supports the argument for no threshold. The activity of a promoter depends on the presence of initiated cells. Promoters are chemicals that induce a hyperplastic response in the target tissue. Hyperplasia is a state of stimulated growth in a tissue, which can be produced in several ways. One mechanism is to kill normal cells in a tissue. Surviving cells are then stimulated to divide in order to "repair" the damage done and restore full organ function. Consequently, if a chemical kills cells in a tissue, the resulting hyperplastic response can stimulate the development of a tumor if initiated cells are present. Obviously, if the chemical is not present at a concentration sufficient to kill cells, a promoting effect cannot result. As a result, the induction of cancer by such a mechanism is usually viewed as a threshold effect. Therefore, if a chemical could be shown to act as a promoter, there would be a substantial difference in the estimation of relative risk for low doses of the chemical.

The relative lack of evidence that chloroform interacts with DNA and its recognized effects on liver and kidney at high doses[32] have caused some investigators[33] to suggest that chloroform acts as a promoter. These arguments are based on the likelihood that many animal tissues (particularly in the mouse) possess cells that have been initiated by other mechanisms (e.g., oncogenic viruses). Consequently, damage to the liver induces a hyperplastic response that is responsible for development of a tumor. Therefore, risk estimates should not involve a linear extrapolation to low response rates as a means of deriving standards. (It should be noted that linear extrapolation was not used in deriving the USEPA's interim standard for THM in drinking water.)

TMD has several ongoing research efforts to help resolve the risk assessment issues associated with chloroform in drinking water. First, there were serious reservations as to whether administration of chloroform by gavage was a good representation of exposure via drinking water. Administering chloroform by means of gavage challenges animals with a concentrated dose over a very short time rather than with a dose spread over a substantial portion of the day as would be the case with human exposure. The concentrated dose certainly would lead to

TABLE 2

General experimental design for distinguishing tumor initiation from tumor promotion

Treatment*	Result	Interpretation
C PPPPPPPPPPPPPP PPPPPPPPPPPPPP	Tumors No tumors	C is tumor initiator
I CCCCCCCCCCCCCCC CCCCCCCCCCCCCC	Tumors No tumors	C is tumor promoter
CCCCCCCCCCCCCC	Tumors	C is whole carcinogen

*C = chemical being tested; P = tumor promoter; I = tumor initiator

TABLE 3

Fat content and pathology of liver in B6C3F1 mice treated with chloroform in drinking water

Chloroform mg/L	Percent Liver Fat*		Number of Animals with Centrilobular Fatty Change*			
	90 Days§	180 Days	30 Days	60 Days	90 Days	Total
0	3.33	5.82	0	0	0	0
200	3.45	7.93†	0	0	0	0
400	3.89‡	6.77	3	0	0	3
600			0	0	0	0
900	4.51‡	7.11†	2	0	0	2
1800	6.36‡	10.40‡	5	0	4	9
2700			6	5	2	13

*Average value for 10 animals at each dose and time
†Statistically significant from control at $P < 0.05$ by ANOVA and t-test
‡Statistically significant from control at $P < 0.01$ by ANOVA and t-test
§Duration of exposure to chloroform at the indicated concentrations

TABLE 4

Activity of haloacetonitriles in screening procedures for mutagenesis and carcinogenesis

Compound	*Salmonella**	DNA Breaks†	Initiation of Tumor in Mouse Skin‡
Chloroacetonitrile	−§	±	+
Dichloroacetonitrile	++	+	±
Trichloroacetonitrile	−	+++	+
Dibromoacetonitrile	−	++	+++
Bromochloroacetonitrile	+++	+	NT**

*Meier et al., personal communication
†Daniel et al., personal communication
‡Bull et al. Toxicologist, 2:326 (1982)
§Indication of negative (−), relative degree of positive (+), or equivocal (±) response
**NT = not tested

higher peak concentrations of chloroform in blood and predictably aggravate necrotic damage to the liver and kidney. Second, the use of corn oil as a vehicle represents a serious metabolic perturbation in the mouse (i.e., 0.2 mL of corn oil per mouse is approximately equivalent to an adult human drinking a full pint of corn oil, all at once, 5 days a week for a lifetime). Third, only two doses were used in the original NCI study. These doses were of such a magnitude that both would be expected to cause liver damage. Consequently, it would not be possible to separate the tumorigenic response from liver damage. With these problems in mind, TMD has sponsored a study that examines doses of chloroform in drinking water ranging down to 200 mg of chloroform/L (approximately equivalent to 20 mg/kg, or about 25 percent of the lowest dose used in the NCI study). The design of this study avoids many of the shortcomings of the earlier NCI study and should provide a better database for establishing the degree of carcinogenic hazard actually associated with chloroform in drinking water. The experimental work has been completed, and the results of the study should be available early in 1983.

In addition, TMD is exploring methods of more precisely defining the extent to which chloroform's carcinogenic effects arise from initiating versus promoting activity. Such effects cannot be clearly differentiated in lifetime studies but can be studied in shorter-term assays that make use of preneoplastic (or precancerous) lesions. Essentially, it must be determined whether the chemical's carcinogenic effect depends on the prior administration of a known initiator, as depicted in Table 2. Such a chemical is known as a promoter. A tumorigenic chemical, which is administered in a single dose or a few doses followed by treatment with a promoting agent, is termed an initiator. Tumorigenic chemicals that are independent of a second agent are called whole carcinogens because they possess both initiating and promoting activity. Results with these tests have been somewhat equivocal to date; a clearly independent promoting activity of chloroform has yet to be proved.[34] These experiments are being repeated and expanded to include the other THMs.

Interim sacrifices conducted during the course of the lifetime study with chloroform do provide some indications of the level of exposure that produces mild but measurable toxic effects. Table 3 indicates the dose-response relationship of chloroform-induced liver damage measured chemically and histologically. Mild indications of fatty change can be seen at the lowest doses employed (200 mg/L), and fatty change becomes histologically apparent in animals receiving 900-2700 mg chloroform/L.

Other chemicals that have recently been recognized as frequent by-products of chlorination are the haloacetonitriles.[35] The health hazards of these chemicals have yet to be characterized. Simmon et al[31] found that dichloroacetonitrile is mutagenic in *Salmonella*. For the reasons indicated above, this can be taken as evidence that a chemical is capable of initiating a tumor. Consequently, TMD has examined chloroacetonitrile, dichloroacetonitrile, trichloroacetonitrile, dibromoacetonitrile, and bromochloroacetonitrile in several short-term bioassays for mutagenic and carcinogenic effects (Table 4). The Ames test (mutagenesis assay in *Salmonella*) confirmed dichloroacetonitrile as a potent mutagen and demonstrated that bromochloroacetonitrile was also active as a mutagen. Chloroacetonitrile and trichloroacetonitrile were clearly negative in this assay, but cytotoxicity of the dibromoacetonitrile was too great to allow evaluation of its mutagenicity. In another assay, which tested for interaction of chemicals with DNA, human lymphocytes were examined for breaks produced in the DNA by a chemical or by the enzymes responsible for repairing DNA damage. In this assay, trichloroacetonitrile was the most active compound followed by dibromoacetonitrile. Dichloroacetonitrile and bromochloroacetonitrile were also clearly active in this assay. Tests of chloroacetonitrile have proved inconclusive. In parallel with these experiments, direct tests for tumor-initiating activity were made in mouse skin.[32] In the latter case, dibromoacetonitrile was clearly the most active initiator, and both chloroacetonitrile and trichloroacetonitrile gave positive results. Data for dichloroacetonitrile proved inconclu-

sive, and bromochloroacetonitrile has not yet been tested.

Unlike the case with chloroform, it is clear that the haloacetonitriles are capable of initiating tumors. However, the tests used are not capable of providing the data necessary for estimating the degree of carcinogenic hazard that results from exposure to these chemicals via drinking water. Lifetime studies in experimental animals are essential for making these determinations.

Complex mixtures of by-products

Concentrates of organic chemicals isolated from the drinking water of several cities have been shown to contain chemicals capable of producing mutations in bacteria and oncogenic transformation of mammalian cells in vitro.[36,37] Such data are taken as indications of the presence of chemicals that possess a potential for being carcinogenic. Concentrates from one of these cities were shown to be capable of initiating tumors in mouse skin.[38] Although the results are not statistically significant, latency to tumor development was uniformly decreased in those animals treated with concentrates from the other cities compared with that for those animals treated with the vehicle alone.

Characteristically, mutagenic chemicals isolated from drinking water in the United States have been direct acting.[36] In other words, the chemicals were capable of producing mutations in the absence of liver enzymes isolated from experimental animals, suggesting that the chemicals did not need to be metabolized before exerting their mutagenic effects. In general, *Salmonella* TA 100 has been the most sensitive strain to the mutagenic effects of these chemicals. The mutagenic activity of these chemicals was substantially reduced by the addition of rat liver enzymes, indicating that these chemicals could be metabolically detoxified. In contrast, activity detected in waters taken from European rivers, such as the Rhine, tends to include chemicals that require metabolic activation.[39]

The observations of several investigators indicate that direct-acting mutagenic chemicals, predominately active in TA 100 and destroyed by rat liver enzymes, are produced by the chlorination of drinking water.[39-43] It is not likely that these effects could be attributed to the formation of volatile organic chemicals, such as THMs, because conditions of these assays were not conducive to the retention of volatile chemicals. Thus, it has generally been concluded that various nonvolatile products of the chlorination process are responsible for the mutagenic effects.

Preliminary experiments investigating the ability of these chemicals to produce mutagenic or carcinogenic effects in intact animals have been conducted with several concentrates (100-400 fold) of settled, coagulated, and filtered water from the Ohio River. These results are summarized in Table 5. The mouse micronucleus assay and bone marrow cytogenetic assay were both found negative in tests for mutagenic effects. Mouse spermhead abnormalities, also often produced by mutagenic chemicals, were not observed to increase with treatments. In these experiments, up to 1.0 mL of the 400-fold concentrate was administered orally to the mice daily for 5 days. The last test, a true bioassay of carcinogenic activity, used a total dose of 1.5 mL of these concentrates per experimental animal. As indicated in Table 5, results with the mouse skin initiation-promotion assay were mixed. As reported in a previous review,[4] one experiment indicated no carcinogenic activity with the nondisinfected water but significant increases in incidence of tumors when the same water was treated with ozone, chlorine, or chloramine. On the other hand, a subsequent experiment indicated some marginal activity in the nondisinfected water and the same or decreased numbers of tumors with disinfected water.[38]

Several interpretations of these results can be made. The first interpretation is that reaction products are not significant in vivo. However, the doses involved in those experiments are small compared with lifetime exposures. Consequently, they do not provide the data needed for a realistic evaluation of risks and do not provide the assurance that no significant carcinogenic hazard exists. A second interpretation is that the samples of organic material were insufficiently concentrated to provide meaningful results with such limited exposures. A third interpretation, drawn primarily from the data for mouse skin, is that the changes in the quality of the source water confound interpretation of these studies unless tests are repeated a sufficient number of times to establish a significant trend. These samples were taken at different times of the year. Parallel experiments[38] demonstrated a considerable variation in the production of volatile and nonvolatile total organic halogen (TOX) at different times of the year.

Little work has been done with by-products of disinfectants other than chlorine specifically in the context of disinfecting drinking water. Zoeteman et al[39] indicated that ClO_2 significantly increased direct-acting mutagenic activity in *Salmonella* strain TA 98, whereas ozonation tended to decrease mutagenic activity. Kool et al[43] also indicated no change or a decrease in activity with ozonation. On the other hand, Gruener[44] observed that ozonation of recycled municipal wastewater resulted in substantial increases in mutagenic activity in *Salmonella* strains TA 98 and TA 100. The basis for these marked differences is not clear but may be related to the high TOC content of the water and the proximity of the sample site to the groundwater recharge pond in the latter case.

The problems cited above have led TMD to seek reasonable models of reaction processes that can be studied under controlled conditions. Results from recent investigations of products formed when disinfectants react with humic and fulvic acids have led researchers to investigate the use of commercially available humic acids as such a model. Stevens et al[45] and Rook[46] have shown that aquatic fulvic acid produces not only THMs in good yield but also alpha chlorinated ketones, chlorinated aldehydes, and chlorinated alcohols. Norwood et al[47] concluded that reactions of aquatic fulvic and humic acids with disinfectants produced similar products and, in addition, documented the formation of small, chlorinated acids.

TMD has begun a systematic investigation of the mutagenic properties of chemicals produced with commercially available humic acids and compared them to products derived from aquatic humic and fulvic acids.[38,48,49] A summary of these results is provided in Table 6. Commercial humic acid* had no activity in the *Salmonella* tester strains but had weak activity in the sister chromatid exchange assay in Chinese hamster ovary cells. In both test systems, the addition of chlorine at pHs below neutrality resulted in substantial increases in mutagenic activity. An essentially identical pattern was observed by using humic and fulvic acids derived from two natural lakes.† The specific mutagenic activity and degree of chlorine incorporation varied among samples, but the commercial humic acid tended to approximate the average of such values rather than having an extreme value.[38]

Analyses of the mixtures produced by chlorination of commercial and Ohio River humic acids by means of GC-MS reveal similar products (Table 7). Chloroform and dichloroacetonitrile are two chemicals clearly identified as by-products of the chlorination of drinking water. In addition, a group of chlorinated acetones, dichloroacetic acid, and trichloroacetic acid were seen. These products are similar to products associated with the use of chlorine in pulp mills.[50,51] As with chlorinated effluents from pulp mills, the mutagenic chemicals produced by chlorination of humic acids are completely destroyed in alkali.[48]

On the basis of the above experiments, it appears that humic acids provide a legitimate model for studying the products of reactions between disinfectants and organic compounds found in drinking waters. Direct application of the data obtained from these studies to actual drinking waters will depend on two ex-

*Fluka (US distributor: Tridom Chemical Co., Hauppage, N.Y.)

†Samples of humic and fulvic acid were provided by R. Christman, University of North Carolina, Chapel Hill, N.C.

TABLE 5

Results with alternatively disinfected drinking water in a number of in vivo bioassays for mutagenic and carcinogenic effects

Samples	Mouse Micronucleus Assay*	Mouse Bone Marrow Cytogenetics Assay*	Mouse Spermhead Abnormalities*	Mouse Skin Initiation-Promotion Assay†		
				Trial 1	Trial 2	Trial 3
Nondisinfected	−‡	−	−	−	−	−
Ozone	−	−	−	+	−	−
Cl_2	−	−	−	+	−	−
ClO_2	−	−	−	−	−	−
NH_2Cl	−	−	−	+	−	−

*Data obtained through contract with Litton Bionetics, Kensington, Md.; up to 1.0 mL of a 400-fold concentrate was administered orally each day for 5 days

†Data from Bull et al.;[32] a total dose of 1.5 mL of up to 400-fold concentrate was administered subcutaneously

‡Indicates positive (+), negative (−), or equivocal (±) result

TABLE 6

Effect of chlorination on mutagenic activity of humic and fulvic acid mixtures

Humic and Fulvic Acid Mixtures	*Salmonella*	Sister Chromatid Exchange in Chinese Hamster Ovary Cells
Commercial humic	−†	+
Commercial humic and HOCl*	+++	+++
Black Lake humic	−	NT‡
Black Lake humic and HOCl	+++	NT
Black Lake fulvic	−	NT
Black Lake fulvic and HOCl	+++	NT
Lake Drummond humic	−	NT
Lake Drummond humic and HOCl	+++	NT
Lake Drummond fulvic	−	NT
Lake Drummond fulvic and HOCl	+++	NT

*HOCl added at 1:1 molar ratio with carbon, which was determined by TOC analysis; solutions contained approximately 1 g TOC/L.

†Indicates whether response was negative (−) or the relative degree of positive (+) response

‡NT = not tested

TABLE 7

*Chemicals identified by GC-MS as by-products of chlorination of humic acids**

Compound	Ohio River Humic Acid	Commercial Humic Acid
Chloroform	+†	+
Dichloroacetonitrile	+	+
Trichloroacetonitrile	−	+
1,1-Dichloropropanone	+	+
1,1,1-Trichloropropanone	+	+
1,1,1,3-Tetrachloropropanone	NA‡	+
1,1,3,3-Tetrachloropropanone	NA	+
Pentachloropropanone	+	+
2,3-Dichloro-2-butanone	+	+
Dichloroacetic acid	NA	+
Trichloroacetic acid	NA	+

*Data from Meier et al.[33]

†A + indicates that humic acid was identified as a by-product of chlorination; a − indicates that humic acid was not detected

‡NA = not analyzed

tensions of these studies: (1) identification of chemicals responsible for the biological activity observed as a result of treatment of humic acids with disinfectant, and (2) demonstration that these chemicals are produced as a result of disinfecting drinking water. The advantage of the model is that it allows controlled experiments to be conducted at higher-than-ambient concentrations, simplifying both the analytical chemistry and biological testing. In addition, the model avoids the artifacts, such as stabilizers used in RO membranes and chemicals derived from resin, associated with the concentration techniques that are absolutely necessary if bioassay methods are to be applied to ambient conditions of drinking water. Once the identity of the products can be established, the development of analytical methods to be applied to ambient conditions will be greatly simplified.

Summary and conclusions

The substantive experimental findings to date are:

- By-products of chlorination that have been identified clearly possess carcinogenic properties. In the case of chloroform, the principal issue appears to be the mechanism by which it produces tumors and the extent to which this affects estimation of risk. The potential hazards of the other THMs and the haloacetonitriles have been identified, but definitive whole animal studies have to be completed.
- Chlorine dioxide, or some reaction product produced in vivo, possesses antithyroid activity. This effect can be clearly demonstrated in monkeys at a concentration of 100 mg ClO_2/L and could pose a substantial problem to the developing animal at lower concentrations.
- A substantial by-product of the use of ClO_2 in high demand waters—ClO_2^-—produces hemolytic anemia. Although probably not critical for normal humans at levels of up to 5 mg/L in drinking water, some concern must be expressed for those segments of the population in which a substantial number of individuals possess exceptional sensitivity to hemolytic agents. Also of concern are those individuals who are on medication or exposed to other chemicals that produce hemolytic anemia.
- Monochloramine possesses mutagenic properties in bacteria and produces changes, which are commonly associated with carcinogens, in the livers of mice. Evaluating the importance of these observations must await the completion of lifetime studies with experimental animals.
- There is too little information to begin evaluating hazards associated with the formation of toxic by-products. This lack of information arises from incomplete understanding of the chemistry, as well as the toxicology, of by-products formed in disinfection. It should be recognized that a disproportionate amount of information exists concerning by-products of chlorination. Care should be taken not to translate this into a premature indictment of chlorine use.
- An area that must be critically evaluated is the formation of by-products on ingestion of drinking water containing a residual disinfectant. Preliminary experiments indicate that by-products similar to those identified in drinking water are formed in vivo at high doses of disinfectant. How this source of by-products compares with by-products formed in water must be examined both in qualitative and quantitative terms.
- Pilot experiments using model substrates, including humic and fulvic acids, indicate that the formation of mutagenic and toxic by-products is substantively affected by water conditions, at least with chlorine. This implies that alteration of the treatment conditions, such as alteration of pH or removal of precursors, may be as important an alternative in treatment as the disinfectant employed.

We are obviously far from completely understanding the nature of health hazards that can arise from the use of disinfectants in drinking water. On the other hand, we do know that there are definite consequences of not disinfecting water that is potentially contaminated by infectious agents. Failure of disinfection is the most common cause of outbreaks[52] of waterborne disease.

Accepting, therefore, the tenent that disinfection of drinking water is a desirable goal, the industry still has the responsibility to minimize the health hazards associated with the use of disinfectants. The first step in this process is to gain some clear appreciation of what

the potential hazards associated with each alternative are. A second step is attempting to quantitate these hazards. Finally, decisions must be made in the complex area of public perceptions of the relative seriousness of competing hazards and economic concerns. It is clear that we are, to a large extent, still struggling with the first of these steps and making progress only in selected instances on the second. It is obviously premature to consider complete resolution of the issues that will surface at the third level of regulatory decision making.

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